Future Fuel: Could Biomass Be the New Petroleum?

Chandra Shekhar
DOI 10.1016/j.chembiol.2011.10.010

Imagine a petroleum alternative that is cheap, plentiful, renewable, and carbon neutral. With the world facing the twin threats of oil scarcity and climate change, exploiting this source should be a no-brainer. Yet such a resource exists and has gone largely untapped until now. The resource is plant biomass, 200 billion tons of which grow every year around the world, with each ton packing as much energy as three barrels of oil. At $50 a ton, it is cheaper than all major energy sources except coal—and cheaper even than clean coal. However, turning this "cellulosic" biomass into liquid fuel such as ethanol has proved technically challenging. Further, incentives for fuel producers and consumers to switch to renewables are lacking. Nonetheless, recent scientific advances and successful pilot projects could help cellulosic biofuels emerge as a viable gasoline alternative. "We are likely to see the first wave of commercial-scale producers emerge over the next few years," says Paul Gilna, head of the US Department of Energy-funded BioEnergy Science Center at the Oak Ridge National Laboratory (ORNL). "I see a strong prospect for cellulosic biofuels."

"It's like a Cambrian explosion of new ideas and technologies…." — Doug Cameron

"First generation" biofuels are made from plant-derived starch, sugar, or fat, all relatively simple to process. The bulk of plant matter, however, is the more intractable material that forms its cell walls. Its main component, cellulose, is a tough, chemically stable, semicrystalline glucose-based polymer that forms fibers like strands of a rope. Coating and connecting these fibers is hemicellulose, another tough, mostly xylose-based polymer. The third—and toughest—part of the cell wall is lignin, a complex, three-dimensional aromatic polymer that permeates and binds the entire structure together, providing strength, durability, and microbe resistance. Armed with these three cell wall polymers, plants have grown tall, survived harsh conditions, and colonized the planet. The flip side of this evolutionary success story, however, is that cellulosic biomass is highly *recalcitrant* to any efforts to break it down, or hydrolyze it, into its component sugars. "Dealing with recalcitrance is key to developing cellulosic biofuels," says Gilna. While cellulosic biomass, unlike starch, sugar, or fat, is too hard for humans to digest, many molds, yeasts and bacteria thrive on it. Cattle and other ruminant animals harbor hordes of biomass-eating microbes in their digestive tracts, as do snails, termites and other wood-boring insects. Together, such microbes decompose 100 billion tons of biomass a year. "We can use the tremendous capability of these organisms in digesting down this complex lignocellulosic material," says Martin Keller of the Energy and Environmental Sciences Directorate at ORNL.

The conventional approach to making "second generation" biofuel is as follows: first, pretreat the biomass with steam, acid, alkali, etc., to loosen lignin, break down hemicellulose, and expose the cellulose; apply a cocktail of cellulase enzymes derived from an organism such as the wood-rotting mold *Trichoderma reesei* to break up the cellulose into its component sugar molecules; ferment these sugars using a batch of brewer's yeast, *Saccharomyces cerevisiae*; and, finally, distill the resulting solution into ethanol. This approach has some drawbacks. Biomass pretreatment is cumbersome and expensive. Digesting cellulose requires 50 pounds or more of costly enzymes per ton of biomass. Hydrolysis of hemicellulose produces xylose, a five-carbon sugar (pentose); common yeasts such as *S. cerevisiae* ferment only six-carbon sugars (hexoses) such as glucose. The pretreatment and hydrolysis stages can release acetates, furans, and other substances that inhibit fermentation. Finally, beyond about 15% concentration, ethanol itself inhibits fermentation. To tackle these issues, researchers have three main strategies: devise better pretreatments, develop plants that make more tractable biomass, and engineer microbes that are better at turning it into biofuel. "Coupling improved feedstock with improved bugs is probably the best way to go forward," says Neal Stewart, a plant scientist at the University of Tennessee in Knoxville.

An ideal biofuel microbe should both hydrolyze and ferment biomass. It should ferment both pentoses and hexoses. It should thrive in the potentially harsh environment of a bioreactor and give maximal yield. It should also be cheap, safe, efficient, and robust. Many molds and bacteria can hydrolyze but not ferment biomass efficiently, while the opposite is true for brewer's yeast. Certain yeasts such as *Pichia stipitis* can ferment pentoses but can't handle inhibitors. Some bacteria such as *Clostridium thermocellum* have evolved an ingenious mechanism called the cellulosome—a highly dynamic, complex arrangement of cellulases and structural proteins—that can dissolve even the most obdurate forms of cellulose such as cotton. However, bacteria are far less adept at making alcohol than yeast. In sum, nature offers an incredible range and diversity of biomass-eating microbes, but none fits the requirements for an ideal biocatalyst. One solution, adopted by University of Pittsburgh at Bradford biologist Om Singh, is to identify promising biofuel microbes and refine their traits using controlled mutations. "We find things directly from nature and utilize them towards the industrial outcome," says Singh.

Could metabolic engineering, used with great success in creating insulin-making

E. coli and other recombinant pharmaceutical organisms, be useful for biofuels as well? Yes, but with one caveat, says University of California, Berkeley, biochemist Michelle Chang. Drug companies typically make small quantities of high-value products, with the emphasis on making complex molecules; process efficiency is secondary. In contrast, biofuels are large-volume, low-cost products that need to be made very efficiently. "Fuel is the hardest thing to make," says Chang. "You have to get close to 100% theoretical yield to make sense from energy and carbon balance perspectives." Getting such a high yield requires thwarting the microbe's inherent nature to grow and multiply rather than transfer carbon to a fuel product. Despite this challenge, Chang, Keller, and other researchers have made significant advances in developing better biofuel microbes. They have coaxed *Saccharomyces* to accept xylose, helped *Pichia stipitis* resist inhibitors, improved *Clostridium thermocellum*'s ability to ferment, and taught *Saccharomyces* how to make cellulosomes to hydrolyze biomass. "Engineering an organism that can both efficiently hydrolyze cellulose and directly convert it to ethanol will be a major achievement on the path to making cellulosic biofuels," says Keller.

One major attraction of second generation biofuels is that they can use a variety of feedstock: hardwood, softwood, agricultural residues, weeds, even waste paper and leaf litter. Researchers are also developing specialized energy crops that promise higher yields and lower costs. Promising candidates for this are herbaceous plants such as miscanthus and switchgrass, and woody species such as poplar and willow. A group led by Richard Dixon, a plant biochemist at the Samuel Roberts Noble Foundation in Ardmore, Oklahoma, has downregulated one of the steps in alfalfa's lignin pathway and thus made the plant more easily digestible as animal feed. This principle could also yield a better biofuel feedstock, Dixon says. Sure enough, downregulating a related lignin gene in switchgrass improves ethanol yield by 30%. In another study, Dixon's group knocked out a gene regulating cell wall synthesis in thale cress to increase the plant's biomass density by 50%. All these plants remain healthy despite their altered cell walls, says Dixon. Other research groups are developing plants with friendlier lignin, less crystalline cellulose, and with hemicellulose composed of glucose rather than xylose. Ideally, "you want to create a plant that can still stand up straight and resist pathogen attack," says Dixon. "Yet when it is ground up and thrown into a pit with a bunch of bugs that have the necessary enzymes, you should get maximum achievable yield even without pretreatment."

Some pretreatment may be unavoidable, however. "Even if lignin could be reduced to zero, the remaining cellulose would be highly crystalline and would still require pretreatment," says Michigan State University researcher Bruce Dale. Dale is the inventor of ammonia fiber expansion (AFEX), an improved pretreatment method that reduces feedstock recalcitrance while minimizing unwanted byproducts.

In parallel with these advances, biofuel companies have been busy turning existing methods into viable industrial processes. In 2004, Ottawa-based Iogen became the first company to set up a cellulosic ethanol demonstration facility, which now makes about 600 gallons a day; in 2009, it became the first to sell this type of fuel at a gas station. "The primary challenge here is economic, as the capital cost is higher than for first generation ethanol," explains Iogen executive Bill Riddick. Enzymes needed for hydrolysis are another major cost component. Supported by a 2001 DoE grant, Denmark-based Novozymes has succeeded in drastically reducing the amount of enzyme needed. An enzyme cost of 50 cents per gallon of ethanol is now achievable, says company executive Cindy Bryant. In 2008, the biofuel company POET set up a pilot plant in Scotland, South Dakota, that makes 20,000 gallons of cellulosic ethanol annually, which costs less than $3 per gallon today. The company is now building a commercial facility in Iowa that will make 25 million gallons per year, says POET executive Wade Robey. Another biofuel company, Abengoa Bioenergia, is building a commercial facility in Hugoton, Kansas, that will make about 23 million gallons a year, says company executive Gerson Santos. A number of other companies have also entered the fray, turning biomass into ethanol, diesel, butanol, gas, and other fuels. "It's like a Cambrian explosion of new ideas and technologies," says Doug Cameron of the biotechnology investment group Alberti Investments. "It'll be a while before it's all sorted out."

For cellulosic biofuel to emerge as a viable petroleum alternative, however, such pioneering efforts will need to be multiplied 1000-fold. Hundreds of billions of dollars of strategic investment will be needed. Unfortunately, such investment is unlikely to happen in today's petroleum-based economy without new government policies. Public funding will be needed for creating the infrastructure to transport and process billion of tons of biomass each year. Most current vehicles can't use more than 10% ethanol; mandates may be needed to coax automobile manufacturers to make flexible fuel vehicles that can. Gas stations and consumers may also need similar inducements to switch from gasoline to ethanol. "At the moment, there is no market differentiation, no market pull, no market push, no regulatory compulsion, no forcing mechanism," said cellulosic policy analyst and former Iogen executive Jeff Passmore at the RETECH 2011 conference held last month by the American Council On Renewable Energy (ACORE) and TradeFair Group in Washington, DC. "Investment in cellulosic ethanol won't happen unless there is long term regulatory stability that delivers price clarity and market certainty."

Chandra Shekhar (chandra@nasw.org) is a science writer based in Princeton, NJ.

